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Lab resource: stem cell line

Generation of an isogenic, gene-corrected iPSC line from a symptomatic 59-year-old female patient with frontotemporal dementia caused by an R406W mutation in the microtubule associated protein tau (MAPT) gene

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ABSTRACT

Frontotemporal dementia with parkinsonism linked to chromosome 17q21.2 (FTDP-17) is an autosomal-dominant neurodegenerative disorder. Mutations in the MAPT (microtubule-associated protein tau) gene can cause FTDP-17, but the underlying pathomechanisms of the disease are still unknown. Induced pluripotent stem cells (iPSCs) hold great promise to model FTDP-17 as such cells can be differentiated *in vitro* to the required cell type. Furthermore, gene-editing approaches allow generating isogenic gene-corrected controls that can be used as a very specific control. Here, we report the generation of genetically corrected iPSCs from a 59-year-old female FTD-17 patient carrying an R406W mutation in the MAPT-gene.

Resource Table:

Name of stem cell line	H237 C3 GC
Institution	Bioneer A/S
Person who created resource	Natakarn Nimsanor, Benjamin Schmid, Mikkel Aabech Rasmussen
Contact person and email	Benjamin Schmid, bsc@bioneer.dk
Date archived/stock date	August 2016
Origin	Human induced pluripotent stem cell line H237 C3
Type of resource	Gene-corrected induced pluripotent stem cells originally derived from skin fibroblasts of a patient with frontotemporal dementia
Sub-type	iPSC line
Key transcription factors	Episomal plasmids containing hOCT4, hSOX2, hL-MYC, hKLF4, hLIN28 and shP53 (Addgene plasmids 27077, 27078 and 27080, all a gift from Shinya Yamanaka; Okita et al., 2011)

1. Resource details

Previously, we have generated an induced pluripotent stem cell (iPSC) line (H237 C3) from a symptomatic, 59-year-old woman carrying an R406W mutation in microtubule-associated protein tau (MAPT) gene. Reprogramming was performed by electroporation with three episomal plasmids encoding hOCT4, hSOX2, hKLF4, hL-MYC, and hLIN28 (Okita et al., 2011; Takahashi et al., 2007). This cell line, termed H237 C3, has previously been described (Rasmussen et al., 2016).

We have generated a gene-corrected clone of H237 C3 using CRISPR/Cas9 technology, where the mutated triplet TGG (tryptophan) was corrected to the wild-type triplet CGG (arginine) using an ssODN (single stranded oligodeoxynucleotide) as homologous template (Fig. 1A). We confirmed by sequencing analysis that the mutated triplet was corrected without further deletions or insertions at the CRISPR cutting site (Fig. 1B). Finally, we confirmed that the cells were still pluripotent after gene-correction (Fig. 1C, D and E) and that they showed a normal karyotype (Fig. 1F).

2. Materials and methods

2.1. CRISPR design

Isogenic gene-corrected controls were obtained using the CRISPRs/Cas9 system in combination with an ssODNs serving as homologous

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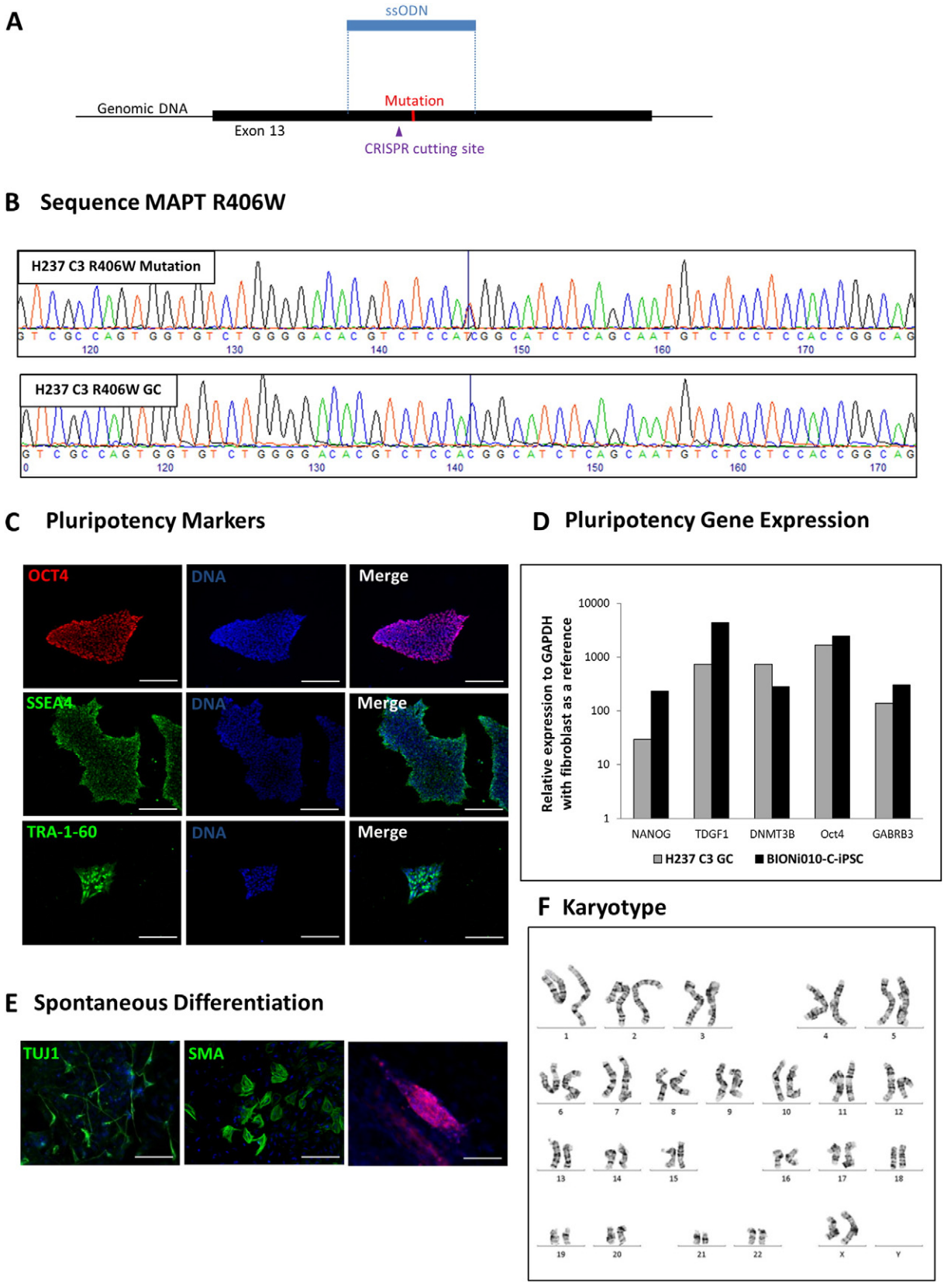

Fig. 1. Characterization of iPSCs. (A) Schematic of the gene editing strategy used to correct the MAPT R406W mutation in exon 13 using the CRISPR/Cas9 system in combination with an ssODN as homologous construct. (B) Sequencing result of the original clone shows a heterozygous C>T mutation (leading to the R406W mutation; upper panel). After gene correction, the mutation was no longer present (lower panel). (C) Immunofluorescence staining for the pluripotency markers OCT4 (red), TRA1-60 (green) and SSEA4 (green), scale bars = 200 μM. (D) Quantitative PCR analysis of pluripotency genes from the gene-corrected line and a previously established iPSC control line BIONi010-C compared to fibroblasts (set to 1). (E) Immunocytochemistry for proteins representative of the three germ layers, Tuj1 (ectoderm), SMA (mesoderm) and AFP (endoderm), after *in vitro* differentiation by embryoid body formation, Scale bars = 100 μM. (F) Representative karyotype of H237 C3 R406W gene-corrected iPSC (46, XX).

template covering the site of the mutation. CRISPRs targeting exon 13 of the MAPT gene were designed at http://crispr.mit.edu/. The CRISPRs were generated in a single plasmid (a gift from Feng Zhang) containing both sgRNA and the Cas9 (pSpCas9(BB)-2A-Puro (PX459); Addgene plasmid #62988) following the protocol from Ann Ran (Ran et al., 2013).

2.2. Nucleofection

iPSCs were cultured on 100 mm dishes coated with Matrigel (Corning Bioscience) in E8 medium and detached using Accutase when they reached a density of 70–90%. A total of 1.5×10^6 cells were co-nucleofected with 8 μg of the CRISPR/Cas9 plasmid PX459, 2 μg of a the pEasyflox I plasmid with a pgk-Neo resistance cassette and 1 μL of 100 μM ssODN using the *P*3 Primary Cell Kit (Lonza) and program CA167 following to the manufacturer's instructions (Lonza). iPSCs were subsequently transferred back to a Matrigel-coated 100 mm dish in E8 medium supplemented with 1:200 diluted Revita cell supplement (Gibco). 24 h post-nucleofection, cells were subjected to neomycin selection for 4 days (2 days 100 nM and 2 days 250 nM) and allowed to recover for a week. Resistant colonies were then picked and expanded for genotyping.

2.3. Genotyping

DNA for genotyping was extracted using the FlexiGene Kit (Qiagen). PCR genotyping was performed using TEMPase Hot Start DNA Polymerase (Ampliqon) according to the manufacturer's instructions at an annealing temperature of 62 °C. The following screening primers were designed covering the R406W mutation in the MAPT gene: Exon 13 forward 5′-CTGGTCTTTCTCTGGCACTT-3′ and Exon 13 reverse 5′-ACCAATTAACCGAACTGCG-3′. The PCR products were digested using *Nco*I for 1 h to detect gene-corrected clones (correction of the mutation leads to loss of NcoI restriction enzyme site). Positive clones were then sequenced using the forward primer to ensure correction of the mutation.

2.4. qRT-PCR analysis of stem cell markers

Total RNA was purified from H237 C3 GC iPSCs, fibroblasts and the iPSC line BIONi010-C (Rasmussen et al., 2014) as a positive control using RNeasy mini kit (Qiagen, Hilden, Germany). Conversion to cDNA was performed with RevertAid First Strand cDNA synthesis kit (Thermo Scientific, Waltham, MA, USA). qPCR analysis was carried out using the TaqMan primers GAPDH Hs03929097_g1, NANOG Hs02387400_g1, OCT4 Hs00999632_g1, TDGF1 Hs02339497_g1, DMNT3B Hs00171876_m1 and GABRB3 Hs00241459_m1 (Thermo Scientific, Waltham, MA, USA; International Stem Cell Banking Initiative). Data was analyzed using the $2-\Delta\Delta Ct$ method relative to GAPDH with fibroblasts as a reference (set to 1).

2.5. In vitro differentiation

iPSCs were dissociated with EDTA (Gibco) and allowed to form aggregates in non-coated cell culture dishes with E8 medium. On day 2, the E8 medium was changed to E6 medium (E6 medium = E8 medium without FGF2 and TGF-β1). On day 7, the aggregates were transferred to matrigel-coated dishes and medium was switched to differentiation medium. For mesodermal differentiation: DMEM/F12 containing 10% FBS, 1% L-glutamine, and 1% non-essential amino acids (all Gibco). For endodermal differentiation: MCDB131-1 containing 0.5% BSA, 0.1% pen/strep, 3 μM CHIR99021 (Selleckchem), 100 ng/mL Activin A (Cell Guidance). For ectodermal differentiation: DMEM/F12 mixed with neurobasal medium in a ratio of 1:1, 1X B27, 1X N2, and 1% L-glutamine (all Gibco), 10 μM SB 431542 and 0.1 μM LDN 193189 (both Selleckchem).

Table 1
Antibodies used for immunochemistry.

	Antibodies and host species	Dilution	Company and catalog number
Pluripotency	Goat anti-OCT4	1:500	Santa Cruz, sc-8628
	Rabbit anti-NANOG	1:100	Millipore, AB5731
	Mouse anti-SSEA4	1:500	BioLegend, 330402
	Mouse anti TRA-1-60	1:500	BioLegend, 330602
	Mouse anti TRA-1-81	1:500	BioLegend, 330702
In vitro differentiation	Mouse anti-smooth muscle actin (SMA)	1:500	Dako, M0851
	Rabbit anti-Alpha-1-fetoprotein (AFP)	1:500	Dako, A0008
	Mouse anti-Beta-III-tubulin (TUJ1)	1:500	Sigma-Aldrich, T8660

Cells were fixed for immunocytochemistry on day 21. ICC analysis was performed with the antibodies against TUJ1, SMA and AFP (Table 1).

2.6. Immunostaining of pluripotency markers

Cells were seeded on matrigel-coated coverslips. After 2 days, the cells were fixed at room temperature with 4% paraformaldehyde in PBS for 10 min, permeabilized with 0.1% Triton X-100 and blocked for unspecific binding with 2% BSA in PBS for 1 h. Immunostaining was performed with primary antibodies against OCT4, NANOG, TRA-1-60, TRA-1-81, and SSEA4 (Table 1). After incubation with the primary antibody, the cells were washed 3 times with PBS and then incubated with fluorescence-conjugated secondary antibodies Alexa fluor 488 (goat *anti*-mouse, 1:1000) or Cyanine 3 (goat anti-rabbit, 1:1000) for 1 h (both Life technologies). The cells were mounted on glass slides with mounting solution-containing DAPI (Life Technologies).

2.7. Verification and authentication

An intact genome was demonstrated by karyotyping using G-banding of 11 mitoses (Fig. 1F). Analysis was performed at the Institute of Medical Genetics and Applied Genomics, University of Tübingen, Germany.

Acknowledgments

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